Steinbrenner Laborsysteme GmbH

primaQUANT 1STEP

primaQUANT 1-Step RT-qPCR Master Mix for Probes

Article	Content
SL-9560_smp	0.5 ml, 50 rxn × 20 µL
SL-9560-1ML	1 ml, 100 rxn × 20 µL
SL-9560-5ML	5 ml, 500 rxn × 20 µL
SL-9560-10ML	10 ml, 1000 rxn × 20 µL
SL-9560-20ML	20 ml, 2000 rxn × 20 µL

Long-Term Storage
at -20°C in the dark

Short-Term Storage
at 4°C in the dark

DESCRIPTION

Our **primaQUANT 1STEP PROBE RT-qPCR Master Mix** for probes is an optimized ready-to-use Master Mix for probe-based assays such as Taqman®, Beacons, MGB or Mediator Probes and can be directly used with both DNA and RNA as a starting material.

It contains a modified and proprietary HotStart DNA Polymerase and Reverse Transcriptase, as well as dNTPS, MgCl2 and other components in optimized concentrations. It provides fast kinetics, a RNA sensitivity of < 10 fg, target amplification even for difficult templates and multiplexing of more than 6 targets.

The **primaQUANT 1STEP PROBE RT-qPCR 2x qPCR Master Mix** contains all components - you just need to add primers and template DNA/cDNA or RNA.

DID YOU KNOW?

For qPCR cyclers requiring ROX as a reference dye, **primaQUANT 1STEP PROBE Master Mix** is also available with ROX (SL-9560R).

VERSION **1.0 – 02 / 2025**

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Recommended Reaction Mixture per Well

BEFORE YOU START

- After thawing, please **invert the Master Mix tube 6-8 times**.
- **Do not vortex** the Master Mix to prevent damage of the enzyme.

Component	Stock Concentration	20 µl Reaction	10 µl Reaction	Final Concentration
2x primaQUANT 1STEP PROBE RT-qPCR Master Mix	2x	10 µl	5 µl	1x
Forward Primer	4 µM	1 µl	0.5 µl	200 nM (100 - 400 nM recommended)
Reverse Primer	4 µM	1 µl	0.5 µl	200 nM (100 - 400 nM recommended)
Probe	8 µM	1 µl	0.5 µl	400 nM (200 - 600 nM recommended)
Template RNA	–	variable	variable	0.1 - 100 ng / Reaction
Steriles Wasser	–	adjust to 20 µl	adjust to 10 µl	–

NOTE

For maximum efficiency and specificity, adjustments of annealing temperature as well as extension time, primer/probe concentration and template concentration may be needed.

CALCULATOR TOOL

Please feel free to download our Excel sheet calculator to calculate the necessary volumes: **calculator.steinbrenner-laborsysteme.de**.

qPCR KnowledgeCenter

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Standard Protocol

NOTE

- For the majority of RT-qPCR assays, standard cycling conditions can be applied.
- However, cycling conditions strongly depend on the primer, probe, amplicon and input material and thus some of these factors might need adjustments.

ONE-STEP RT-QPCR WITH ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		10 minutes	50°C
Initial Denaturation		1 - 3 minutes	92°C - 95°C
25 - 40 cycles	Denaturation	5 seconds	92°C - 95°C
	Annealing	5 seconds	60°C depending on primer
	Extension	5 - 10 seconds	72°C

ONE-STEP RT-QPCR PROTOCOL WITHOUT ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		10 minutes	50°C
Initial Denaturation		1 - 3 minutes	92°C - 95°C
25 - 40 cycles	Denaturation	5 seconds	92°C - 95°C
	Annealing / Extension combined	5 - 20 seconds	60°C depending on primer

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Ultra-fast Protocol

NOTE

- Ultra-fast cycling conditions highly depend on the ramping rate of your qPCR cycler, primer, probe, amplicon and input material and thus might need adjustments.
- Ultra-fast cycling conditions can be applied for the majority of qPCR assays out-of-the box, provided that your primer/probe sets do not show unspecific binding.

ONE-STEP RT-QPCR PROTOCOL WITH ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		5 - 10 minutes	50°C
Initial Denaturation		1 minute	92°C - 95°C
25 - 40 cycles	Denaturation	1 second	92°C - 95°C
	Annealing	1 - 5 seconds	60°C depending on primer
	Extension	1 second	72°C

ONE-STEP RT-QPCR PROTOCOL WITHOUT ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		5 - 10 minutes	50°C
Initial Denaturation		1 minute	92°C - 95°C
25 - 40 cycles	Denaturation	1 second	92°C - 95°C
	Annealing / Extension combined	1 - 5 seconds	60°C depending on primer

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Applications

Probe-based quantitative PCR with RNA Input

Probe-based quantitative PCR with cDNA/DNA Input

Multiplex RT-qPCR Assays for up to 8 colors

QUALITY CONTROL

Our **primaQUANT 1STEP PROBE 2x qPCR Master Mix** is subject to strict quality controls. Each lot is tested in a probe-based qPCR with cDNA and DNA input and must conform to our quality control chart.

Enzyme **purity and homogeneity of > 98 %** is validated by Bioanalyzer SDS protein electrophoresis.

The primaQUANT 1STEP PROBE 2x qPCR Master Mix is free of:

- RNA
- DNA
- RNase
- DNAse
- Endo- & Exonuclease activity

FURTHER INFORMATION

For more information, please visit our website: **www.steinbrenner.de**

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Further Products

Products that may also interest you

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